

CLAIMS

1. An immunoassay method in which a target substance in a specimen is assayed by the use of two types of antibodies, and which comprises

using the two types of the antibodies, i.e., a first antibody and a second antibody which have the following properties: (i) the first antibody has affinity for the target substance and a competitive substance, (ii) the first antibody has a higher affinity for the target substance than for the competitive substance, (iii) the second antibody has a higher affinity for the competitive substance than for the target substance, and (iv) the affinity for the competitive substance of the second antibody is higher than the affinity for the target substance of the first antibody,

bonding the target substance and the competitive substance in the specimen to the first antibody and second antibody adsorbed on a carrier, and then

measuring the level of the bonded target substance to assay the target substance in said specimen.

2. An immunoassay method according to claim 1, wherein furthermore, the affinity for the target substance of the second antibody is higher than the affinity for the competitive substance of the first antibody.

3. An immunoassay method according to claim 1 or 2, wherein the target substance is an intact enzyme and the measurement of the level of the target substance bonded is the measurement of the enzymatic activity of said intact enzyme.

4. An immunoassay method according to claim 3, wherein the competitive substance is a substance not having said enzymatic activity.

5. An immunoassay method according to claim 3 or 4, wherein the competitive substance is an enzyme degradation product.

6. An immunoassay method according to any one of claims 3 to 5, wherein the intact enzyme is tartrate resistant acid phosphatase 5b (TRACP 5b).

7. An immunoassay method according to any one of claims 1 to 6, wherein the carrier is an insoluble solid support.

8. An immunoassay method according to any one of claims 1 to 7, wherein the carrier on which the first antibody is adsorbed is a solid support, and the second antibody is adsorbed on a carrier dispersed in a solution or is dissolved.

9. A kit for immunoassay of a target substance in a specimen by the use of two types of antibodies, which comprises

the two types of the antibodies, i.e., a first antibody and a second antibody which have the following properties: (i) the first antibody has

affinity for the target substance and a competitive substance, (ii) the first antibody has a higher affinity for the target substance than for the competitive substance, (iii) the second antibody has a higher affinity for the competitive substance than for the target substance, and (iv) the affinity for the competitive substance of the second antibody is higher than the affinity for the target substance of the first antibody.

10. A kit according to claim 9, wherein the first antibody and the second antibody are adsorbed on a carrier.

11. A kit according to claim 9 or 10, wherein the first antibody is adsorbed on a solid support and the second antibody is adsorbed on a carrier dispersed in a solution or is dissolved.

12. A marker molecule for diagnosing bone disease, comprising a fragment of tartrate resistant acid phosphatase 5b (TRACP 5b) having a molecular weight of approximately 5580 Da, 5795 Da, 6860 Da or 7075 Da.